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GTP-BINDING PROTEINS IN A CYANOBACTERIUM Anabaena cylindrica

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GTP-binding proteins were detected in a crude extract containing membrane components of Anabaena cylindrica. The crude extract was treated with 1 % Lubrol PX and was fractionated by gel filtration. The binding of [^{35}S]GTPγS to GTP-binding proteins was prevented in the presence of 0.1 mM GTP and in the presence of 0.1 mM ATP. Six fractions of these GTP-binding proteins, tentatively designated GA1 to GA6, were ADP-ribosylated by pertussis toxin. GA3, GA4 and GA5 had Km values of 10, 60 and 7 nM, respectively. The molecular weights of some of these GTP-binding proteins were reduced after being labelled with [^{35}S]GTPγS.

Cyanobacteria are thought to be the first organisms to have aquired higher plant-type oxygen-evolving photosynthesis (1). Light is used as an energy source for photosynthesis and also seems to have some signal function. We have demonstrated that the intracellular concentration of cyclic 3',5'-AMP decreases rapidly when cells subjected to darkness for 30 min are irradiated with white light (Ohmori, M. et al., submitted). In a monocotyledonous plant, Lemna paucicostata, far red light irradiation of plants previously subjected to 4 h of darkness resulted in a change in the ratio of the concentrations of cyclic 3',5'-AMP to cyclic 3',5'-GMP (2). Red light irradiation of germinating spores of a fern, Lygodium japonicum, resulted in a decrease in the concentration of cyclic 3',5'-AMP (3). In L. paucicostata we detected at least four

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species of GTP-binding proteins. Red light or far red light changed the binding rates of [^{35}S]GTPγS to GTP-binding proteins (4,5). These results indicate that the fundamental process of light perception in green plants is very similar to that of retinal rod outer segments of frog eye (6), in which the photoactivated rhodopsin stimulates the conversion of transducin-GDP to transducin-GTP. Transducin-GTP readily releases Tα-GTP from β and γ subunits (7). Tα-GTP removes the γ subunit of photoactivated cyclic phosphodiesterase resulting in the activation of the enzyme, and thus the level of cyclic 3',5'-GMP drops rapidly. Despite these similarities in the process of photoperception in plants and animals, the GTP-binding proteins in Pisum showed unusual properties in their affinity to ATP (8). GTP-binding proteins from animals and from the filamentous fungus Neurospora do not show affinity to ATP (9). In the present paper we report the detection of several species of GTP-binding proteins in a cyanobacteria A. cylindrica, which are very similar to those observed in higher plants.

EXPERIMENTAL

Growth of bacteria: Cells of A. cylindrica were grown to late logarithmic phase under white light (3 klux) in a nitrogen-free culture medium at 28 °C as reported previously (10). The cells were collected and washed once with fresh culture medium by centrifugation. The cell paste was stored at -80 °C in darkness.

Preparation of crude extract: The cell paste was allowed to melt and, after thawing, 2.6 g was suspended in 16 ml of an extraction buffer (4). The cell suspension was chilled in ice-water and was then sonicated 5 times with a sonic probe (Branson, Model 200) at full power for 1 min of 1 min intervals. The disruption of the cells was monitored directly by observation with a light microscope. The cell homogenate was centrifuged at 5,000 x g for 10 min and 2 °C. The supernatant (17 ml) was divided into 1 ml aliquots and stored at -80 °C. The crude extract contained 8.4 mg protein/ml.

Assay for GTP-binding activity: The assay for the binding of [^{35}S]GTPγS (Guanosine 5'[γ-thio] triphosphate, [^{35}S]-; 1013 Ci /mmol, NEG-030H) to GTP-binding protein was carried out as previously described (4) using a Norit solution. Protein was measured using a Bio-Rad protein assay kit.

ADP-ribosylation of GTP-binding protein: ADP-ribosylation of GTP-binding proteins by pertussis toxin (Kaken Pharmaceutical Co.) was performed as previously described (11) using [^{32}P]NAD (NEG-023X: 0.504 mmol 0.5 mCi^{-1}).

RESULTS AND DISCUSSION

Detection of GTP-binding proteins: The crude extract containing plasma membrane and thylakoids was diluted two-fold with extraction buffer and the mixture was then made 1 % with Lubrol PX. After incubation of the mixture at 25 °C for 10 min, it was loaded onto a Sephadex G-100 gel column. The binding of GTP-binding proteins to [^{35}S]GTPγS, and ADP-ribosylation of proteins by pertussis toxin with [^{32}P]NAD for each fraction is shown in Fig. 1a. In terms of binding of [^{35}S]GTPγS, a shoulder of activity occured in fractions 13-15 and 16-18; the peak of activity was in

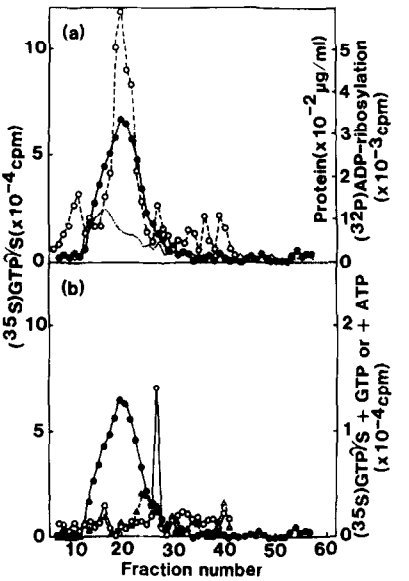

Fig. 1. Gel filtration through a Sephadex G-100 column of a crude extract treated with 1 % Lubrol PX. The crude extract containing membrane components (8.4 mg protein) (1 ml) was diluted with an equal volume of extraction buffer and the mixture was made 1 % with Lubrol PX and incubated at 25 °C for 10 min. After chilling in ice-water it was loaded onto a Sephadex G-100 column (2×32 cm) equilibrated with 20 mM Tris-HCl, pH 7.2, 20 mM NaCl, 25 mM $MgCl_2$, 0.1 % Lubrol PX and 1 mM phenyl methylsulfonyl fluoride (buffer A), eluted with the same buffer, and 2.7 ml fractions were collected. (a) Binding of [^{35}S]GTPγS to GTP-binding protein (—●—), ADP-ribosylation of proteins (---○---) and protein concentration (·····). (b) Binding of [^{35}S]GTPγS to GTP-binding protein in control experiment (—●—), in the presence of 0.1 mM GTP (—○—) and in the presence of 0.1 mM ATP (---△---).

fractions 19-21; fractions 22-24 formed a small shoulder; fractions 26-28 also formed a shoulder, and fractions 30-32 showed a small peak of activity. Proteins in all of these fractions were ADP-ribosylated by pertussis toxin with [^{32}P]NAD. These GTP-binding proteins were tentatively designated as GA1, GA2, GA3, GA4, GA5 and GA6.

To assay the binding of [^{35}S]GTPγS to GTP-binding protein, 0.1 mM GTP or 0.1 mM ATP was added to the reaction mixture with 5 nM (1μCi) of [^{35}S]GTPγS. Competitive inhibition of binding of [^{35}S]GTPγS to the GTP-binding protein was then determined. The bindings of GA1, GA2, GA3, GA4, GA5 and GA6 to [^{35}S]GTPγS in the presence of 0.1 mM GTP were 7.3, 4.1, 1.0, 3.4, 50.3 and 43.0 %, respectively, and in the presence of 0.1 mM ATP were 8.3, 2.9, 1.5, 5.6, 14.5 and 27.4 %, respectively. Since the binding activity of GA6 to [^{35}S]GTPγS was so low further precise work is needed to determine these values. All of the GTP-binding proteins showed affinities to ATP comparable to GTP, so that these GTP-binding proteins may also be involved in ATP binding. Similar results were obtained in Lemna (Furukawa, K. et al., submitted), and in etiolated epicotyls of pea seedlings (8). The competitive inhibition of binding of GA5 to [^{35}S]GTPγS by 0.1 mM ATP was more than that by 0.1 mM GTP. GA5 was assumed to be an ATP-binding protein rather than a GTP-binding protein.

Fractions designated GA3, GA4 and GA5 were pooled and the Km values of the GTP-binding proteins in the fraction were determined. The Km values for GA3, GA4 and GA5 were 10, 60 and 7 nM, respectively. These values are close to those observed for Gs and Gi of adenylate cyclase and for those in transducin (14).

Gel filtration analysis of reaction products: The crude extract (1.0 ml) was diluted with an equal volume of the extraction buffer and was then mixed with 0.5 ml of a 5-fold concentrated

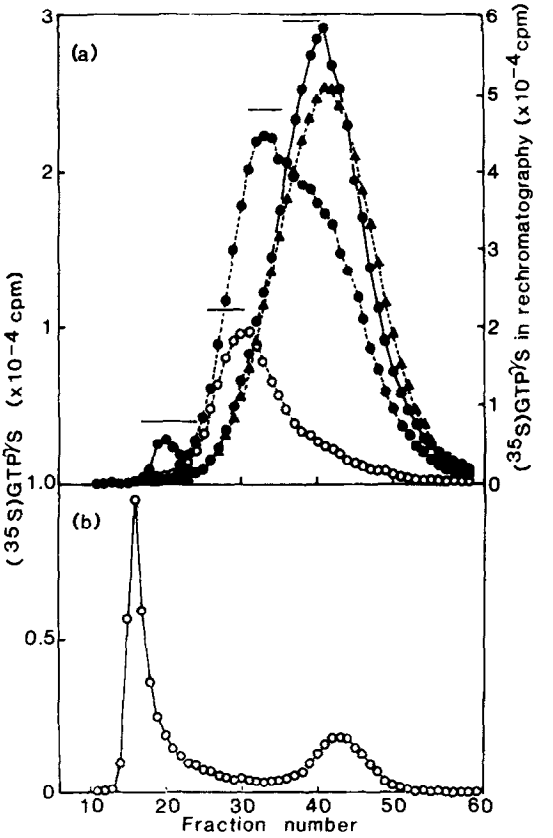

Fig. 2. Sephadex G-25 and G-100 gel filtration analysis of the reaction product of [^{35}S]GTPγS and the crude extract. (a) The reaction product, obtained as described in the text, was loaded onto a Sephadex G-25 gel column (2 ×32 cm) equilibrated with buffer A, eluted with the same buffer, and 2.7 ml fractions were collected. Fractions under the bars were pooled and rechromatographed. Radioactivity was in each 0.1 ml fraction (—●—). Fractions 26-30 were pooled and 4.8 ml was loaded onto the same Sephadex G-25 column. Radioactivity in each 2.5 ml fraction (---○---). The same procedure was applied to fractions 31-35 (---●---) and fractions 36-40 (---▲---). (b) Fractions 17-24 were pooled, loaded onto a Sephadex G-100 column (2×32 cm) and fractionated as described above. Radioactivity in each 2.5 ml fraction (—○—).

reaction mixture containing 10 μCi of [^{35}S]GTPγS. The mixture was incubated at 25 ^{o}C for 1 min. After chilling it in ice water, 2.5 ml of a 2-fold concentrated quenching mixture and 0.5 ml of 10 % Lubrol PX were added, and the mixture was then further incubated for 10 min at 25 ^{o}C. It was then chilled in ice water and was loaded onto a Sephadex G-25 gel column. The elution profile is shown in Fig. 2a. Void volume fractions 17-24, indicated by the bars, were collected and 4.8 ml of the pooled eluate was loaded

onto a Sephadex G-100 gel column (Fig. 2b). The first large peak contains GA1 and GA2 and the very small peak at fraction 30 may correspond to GA6. A radioactivity peak from fractions 38-48 indicates the release of α-subunit of a GTP-binding protein. Re-chromatography of the pooled fractions, 26-30, 31-35 and 36-40 through a Sephadex G-25 gel column also showed at least two peaks of radioactivity at fractions 29-32 and 30-33 from the pooled fractions 26-30 and 31-35, respectively (Fig. 2a). Since free [^{35}S]GTPγS was eluted at fractions 41-42, these two peaks represent [^{35}S]GTPγS bound to the subunit of GTP-binding proteins. The binding of [^{35}S]GTPγS to GTP-binding protein may be irreversible, since incubation of the reaction mixture with a quenching mixture containing 0.1 mM GTP did not affect the amount of [^{35}S]GTPγS bound to GTP-binding proteins. Similar reductions in the molecular weights of the GTP-binding proteins bound to [^{35}S]GTPγS were reported from Lemna (5) and from etiolated epicotyls of pea seedlings (8). These GTP-binding proteins in A. cylindrica, showing strong affinities to ATP, seem to have the general characteristics of GTP-binding proteins found in animals, Neurospora (9) and green plants (5,8), and may represent ATP-GTP-binding proteins. Since the evolutionary origin of cyanobacteria predates that of higher plants and animals, ATP-GTP-binding proteins may be a prototype of GTP-binding proteins, which function as transducers of external stimuli.

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REFERENCES

1. Nicholls, D. G. (1982) Bioenergetics: An Introduction to the Chemiosmotic Theory. Academic Press. New York.
2. Hasunuma, K., Shinohara, Y., Funadera, K. and Furukawa, K. (1987) XIV Intern. Bot. Congr. Abstr. 142.
3. Sugai, M., Yamada, K., Manabe, K. and Hasunuma, K. (1987) XIV Intern. Bot. Congr. Abstr. 137.
4. Hasunuma, K. and Funadera, K. (1987) Biochem. Biophys. Res. Commun. 143, 908-912.
5. Hasunuma, K., Furukawa, K., Funadera, K., Kubota, M. and Watanabe, M. (1987) Photochem, Photobiol. 46, 531-536.
6. Stryer, L., Hurley, J. B. and Fung, B. K-K. (1981) Curr. Top. Membr. Transp. 15, 93-108.
7. Bennet, N. and Dupont, Y. (1985) J. Biol. Chem. 260, 4156-4168.
8. Hasunuma, K., Furukawa, K., Tomita, K., Mukai, C. and Nakamura, T. (1987) Biochem. Biophys. Res. Commun. 148, 133-139.
9. Hasunuma, K., Miyamoto-Shinohara, Y. and Furukawa, K. (1987) Biochem. Biophys. Res. Commun. 146, 1178-1183.
10. Ohmori, M. and Hattori, A. (1978) Arch. Microbiol. 117, 17-20.
11. Sternweis, P. C. and Robishaw, J. D. (1984) J. Biol. Chem. 259, 13806-13813.
12. Hughes, S. M. (1983) FEBS Letters, 164, 1-8.